

Project No. _____
Book No. _____ TITLE _____

116

12/1/94

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Purpose: To try new primers again with PCR
to get rid of mispriming optimization of Mg

used: KlenTag buffer w/o Mg added Mg later, different conc
5 µl

1 Unit Klg
1 µl primer
200 ng template
200 µM dNTP

included:
primer 1 alone
2 "
w/o primer
w/o Mg as controls

µl	1	1.5	2	2.5
5	7.5	10	12.5	
45	42.5	40	37.5	3
50				
1				
5 µl / rx				

Cycling: 94° 3'
30 (94° 30", 56° 30", 72° 3')
72° 60"
4°

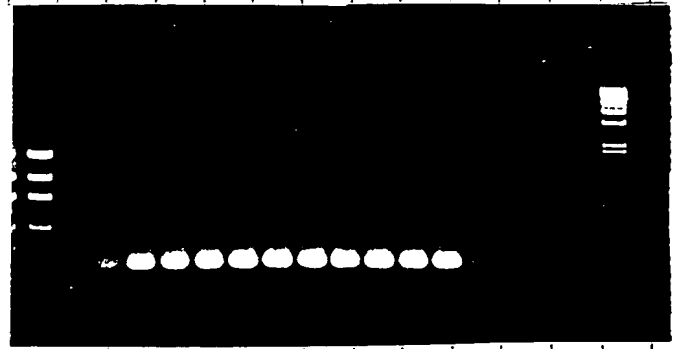
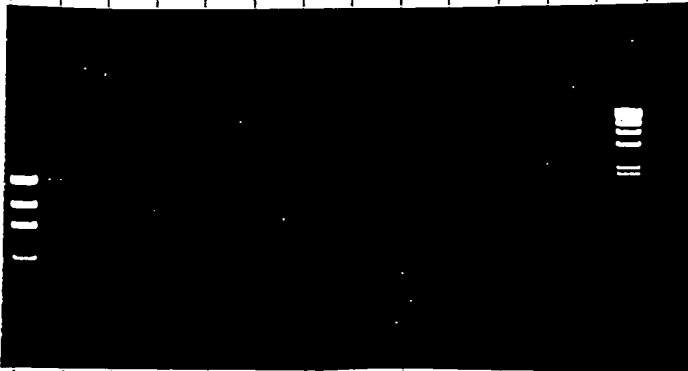
10 x buffer	60
dNTP	120
Template	2.4
enzyme	2.4
primer 1	6.0
2	6.0
H ₂ O	45 / 1.2
	Mg
45 µl +	5 µl

Did the same with new
du primers

2720
2729

no. 7 + 10.6 primer 1 & 2
1441.9 H₂O

w/o primer assembled separately



Witnessed & Understood by me,

[Signature]

Date

12/1/94

Investigated by

Recorded by

[Signature]

Date

12/2/94

T Page

g N _____

Result: no product in both sets of reactions.

more primer dimer with new dU primers?

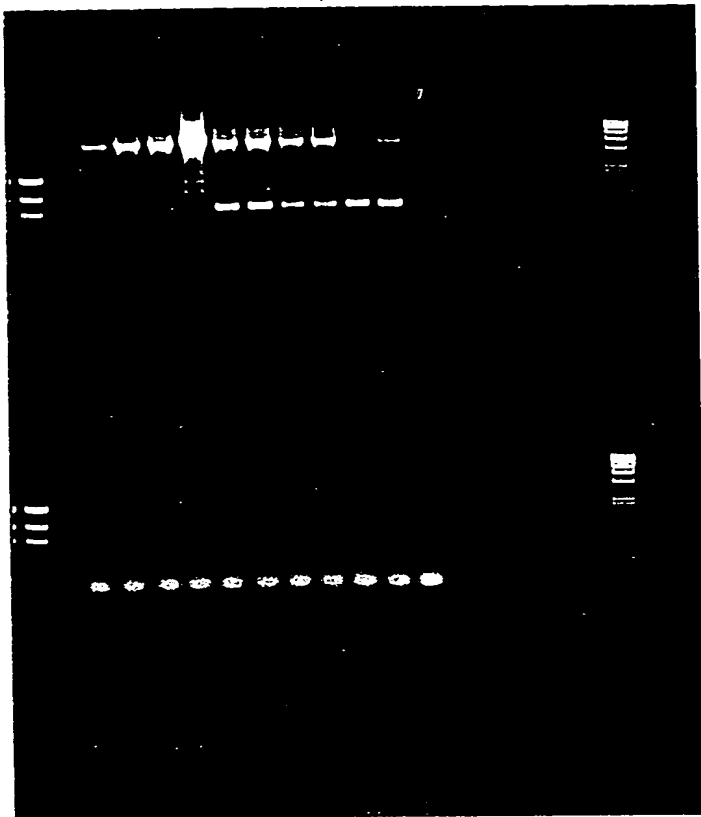
10 of Tag, 200 pg Template for 6.1 Kb - enzyme might not be enough. Did tag pg 102 - got products in KlenTag buffer but not of mispriming was observed.

Since it didn't work & no products were seen don't know about the effect of Mg.

repeated the expts again with DeepVent + Tag (0.01 + 1.0)

Result:

1d dU 1 1.5 2 2.5 3 mM Mg



New dU

Once again new dU primer didn't work even with this enzyme cocktail

- Less Mg = less mispriming
the lower band almost disappears

- 3 mM has product than 1.5 - 2.5 mM

- Again with dU - new primer more primer dimer

* * Try with more enzyme
Tag - 2U

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sed & Understood by me,

Date

Invented by

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Recorded by

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12/2/94